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TITLE: Targeting the APOBEC3B-Induced Mutation Showers in Breast Cancer

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14. ABSTRACT

Genomic instability is one of the hallmarks of breast cancer and fuels tumor development as well as metastasis. Recent cancer genomics studies have revealed the cytosine deaminase APOBEC3B is commonly overexpressed in breast cancers, suggesting that it may be an important cause of genomic instability in the context of breast cancer cells. In our studies, we have established cell lines that inducibly express APOBEC3B and the closely related APOBEC3A. Using these cell lines, we found that overexpression of APOBEC3A and APOBEC3B indeed induced genomic instability. Importantly, we found that cells overexpressing APOBEC proteins are highly sensitive to inhibitors of ATR, a master regulator of DNA repair. In contrast, inhibitors of ATM and DNA-PK, two other regulators of DNA repair, did not affect the survival of APOBEC overexpressing cells, suggesting that ATR has a unique role in the repair of APOBEC induced DNA damage. In addition to APOBEC inducible cell lines, we have identified cancer cell lines that express high or low levels of APOBEC. Our preliminary results suggest that high levels of APOBEC in cancer cells render them hypersensitive to ATR inhibitors.

15. SUBJECT TERMS

APOBEC, ATR, DNA repair, genomic instability, cancer therapy

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1. Introduction

Genomic instability is a hallmark of cancer, and it provides an opportunity for cancer therapy. Recent studies have shown that the genomes of breast cancer cells contained particularly high levels of clustered mutations. In addition, overexpression of the APOBEC family proteins, which are DNA cytosine deaminases, has been linked to this unique type of genomic instability in breast cancer cells. The goal of this project is to establish cellular models of APOBEC overexpressing cancer cells. Using these models, we will test if the ATR kinase, a master regulator of DNA damage responses, is critical for the survival of cancer cells in the presence of APOBEC-induced DNA damage. Furthermore, we will investigate whether and how ATR regulates DNA repair pathways to cope with APOBEC-induced DNA damage in cancer cells. Finally, using the APOBEC overexpressing cellular models, we will perform a chemical screen for compounds that selectively kill cancer cells with high levels of APOBEC. These studies may ultimately allow us to develop new therapeutic strategies to eliminate APOBEC-overexpressing cancer cells, particularly breast cancer cells.

2. Keywords

APOBEC, ATR, DNA repair, genomic instability, cancer therapy

3. Accomplishments

3a. What were the major goals of the project?

Task 1. Generation of breast cancer cell lines with high or low levels of APOBEC3B (90% complete).

Task 2. Examine if APOBEC3B-expressing cells are defective for HR using the RAD51 foci assay and the DR-GFP assay (100% complete).

Task 3: Examine if APOBEC3B-expressing cells are sensitive to ablation of NHEJ or MMEJ using siRNAs targeting specific repair proteins (50% complete).

Task 4: Examine if APOBEC3B-expressing cells are sensitive to inhibition of the replication checkpoint using the cell viability assay (100% complete).

Task 5: Examine if APOBEC3B-expressing cells are sensitive to inhibition of UNGs using siRNAs and the cell viability assay (100% complete).

Task 6: Examine if APOBEC3B-expressing cells are sensitive to inhibition of MMR using siRNAs and the cell viability assay (50% complete).

Task 7: Generate APOBEC3B-high and –low cell lines with different colors (50% complete).

Task 8. Conduct a chemical screen for compounds that specifically kill APOBEC3B-high cells (not initiated)

Task 9: Validation of compounds (not initiated)

Task 10: Determine the mechanism of action (not initiated)

3b. What was accomplished under these goals?

Task 1. Generation of breast cancer cell lines with high or low levels of APOBEC3B.

To investigate how to kill APOBEC overexpressing cells selectively, we sought to establish cell lines that inducibly express APOBEC proteins. We have now successfully overexpressed APOBEC3A and APOBEC3B in several cell lines. Due to their cytosine deaminase activity, E. coli cannot tolerate expression of APOBEC proteins. We have tried a number of strategies to overcome this problem. Finally, we found that inclusion of an intron in the coding sequences of APOBEC3A and 3B allowed propagation of these plasmids in E. coli. When these plasmids are introduced to human cells, the intron is spliced out and APOBEC proteins are successfully expressed. We have now established cell lines that inducibly express APOBEC3A and 3B (Fig. 1).

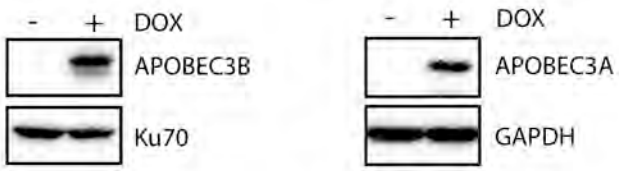

Fig. 1. Generation of cell lines that inducibly express APOBEC proteins. Lentivirus expressing APOBEC3A and APOBEC3B were generated, and used to infect multiple cancer cell lines. Upon induction with Dox, APOBEC3A and APOBEC3B proteins were detected by Western blot using specific antibodies. Ku70 and GAPDH were used as loading controls.

Task 2. Examine if APOBEC3B-expressing cells are defective for HR using the RAD51 foci assay and the DR-GFP assay.

We speculated that APOBEC overexpression might interfere with HR because of the deamination of cytosine in ssDNA. During our studies, we realized that our original strategies had two caveats: (1) RAD51 focus formation only monitors the early steps of HR; (2) the DR-GRP reporter cannot be easily introduced to our APOBEC inducible cell lines. We have accomplished this task using an alternative approach. Defects in HR are known to render cells hypersensitive to PARP inhibitors. To examine if APOBEC overexpressing cells are HR defective, we tested the PARP inhibitor sensitivity of cells after APOBEC induction. Our results showed that cells overexpressing APOBEC were not hypersensitive to PARP inhibitors (Fig. 2), suggesting that they are not defective of HR.

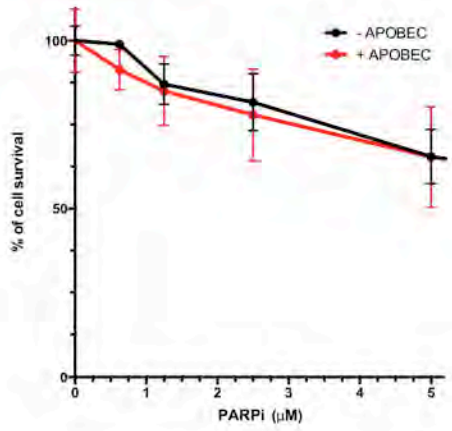

Fig. 2. Expression of APOBEC does not affect homologous recombination. APOBEC inducible cell line was treated with Dox to induce APOBEC expression, or with DMSO as a control. Cells expressing APOBEC or control cells lacking APBEC were treated with increasing concentrations of PARP inhibitor. The survival of cells was analyzed by CellTiter-Glo.

Task 3: Examine if APOBEC3B-expressing cells are sensitive to ablation of NHEJ or MMEJ using siRNAs targeting specific repair proteins.

APOBEC induced cytosine deamination may interfere with NHEJ or MMEJ. DNA-PK is known to be a key regulator of NHEJ. To examine if APOBEC overexpressing cells are sensitive to ablation of NHEJ, we tested the sensitivity of cells to DNA-PK inhibitors after APOBEC induction. Our results showed that cells overexpressing APOBEC were not hypersensitive to DNA-PK inhibitors (Fig. 3), suggesting that they are not dependent on NHEJ for survival.

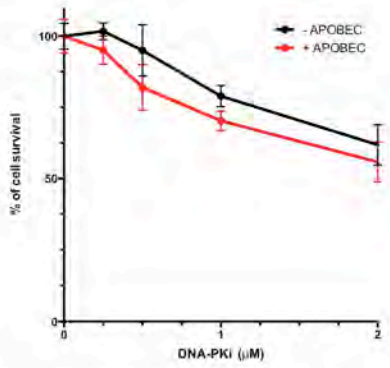

Fig. 3. Expression of APOBEC does not render cells sensitive to inhibition of NHEJ. APOBEC inducible cell line was treated with Dox to induce APOBEC expression, or with DMSO as a control. Cells expressing APOBEC or control cells lacking APBEC were treated with increasing concentrations of DNA-PK inhibitor. The survival of cells was analyzed by CellTiter-Glo.

Task 4: Examine if APOBEC3B-expressing cells are sensitive to inhibition of the replication checkpoint using the cell viability assay.

We hypothesized that APOBEC mediated cytosine deamination creates replication stress and renders cells reliant on the replication checkpoint for survival. ATR is a master regulator of the replication checkpoint. To examine if APOBEC overexpressing cells are sensitive to inhibition of replication checkpoint, we treated cells with ATR inhibitors after the induction of APOBEC, and analyzed their viability in 6 days. Our results showed that APOBEC overexpressing cells are more sensitive to ATR inhibition than their uninduced counterpart (Fig. 4), suggesting that APOBEC expression indeed renders cells reliant on the replication checkpoint.

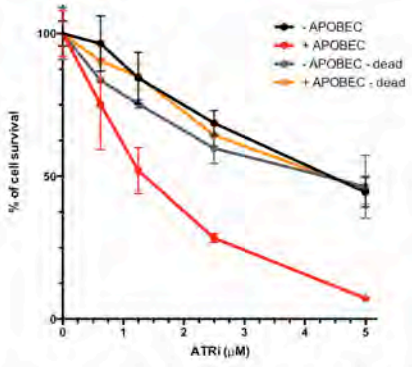

Fig. 4. Expression of APOBEC renders cells hypersensitive to ATR inhibition. APOBEC inducible cell line was treated with Dox to induce APOBEC expression, or with DMSO as a control. The catalytically dead APOBEC protein (APOBEC-dead) was also induced as a negative control. Cells expressing APOBEC or control cells expressing APOBEC-dead or lacking APBEC were treated with increasing concentrations of ATR inhibitor. The survival of cells was analyzed by CellTiter-Glo.

Task 5: Examine if APOBEC3B-expressing cells are sensitive to inhibition of UNGs using siRNAs and the cell viability assay.

Deaminated cytosine is known to be processed by basic excision repair through UNG. To examine if APOBEC overexpressing cells are sensitive to inhibition of UNG, we induced APOBEC expression and treated cells with UNG siRNA. Surprisingly, knockdown of UNG did not induce DNA damage as indicated by γH2AX levels. Furthermore, knockdown of UNG reduced the levels of γH2AX in APOBEC overexpressing cells after ATR inhibition (Fig. 5). These results suggest that APOBEC expression does not render cells sensitive to inhibition of UNG.

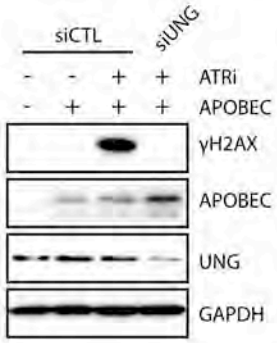

Fig. 5. UNG depletion does not increase the genomic instability in cells expressing APOBEC. Cells expressing APOBEC were treated with UNG siRNA. The levels of genomic instability was monitored by γH2AX, a marker of DNA breaks. Depletion of UNG did not increase genomic instability in cells expressing APOBEC. In contrast, ATR inhibitor (ATRi) drastically increased the genomic instability in APOBEC expressing cells.

Task 6: Examine if APOBEC3B-expressing cells are sensitive to inhibition of MMR using siRNAs and the cell viability assay.

APOBEC mediated cytosine deamination may create a dependence on MMR for cell survival. We will test this hypothesis using siRNAs targeting MMR proteins. These experiments are still in process. Reagents have been made for these experiments. We will present results in the next report.

Task 7: Generate APOBEC3B-high and –low cell lines with different colors.

Cell lines overexpressing APOBEC or not will be labeled with different colors and used in the chemical screen for compounds that selectively kill APOBEC overexpressing cells. We have generated retrovirus expressing GFP and mCherry. We are still in the process of making the cell lines stably expressing these color reporters. We will present result in the next report.

Task 8: Conduct a chemical screen for compounds that specifically kill APOBEC3B-high cells.

We are still in the preparation stage for the chemical screen. We will perform this screen after we confirm the APOBEC inducible cell line and control cell line are stably labeled with GFP and mCherry, respectfully.

Task 9: Validation of compounds.

This task is proposed for years 2-3.

Task 10: Determine the mechanism of action.

This task is proposed for year 3.

3c. What opportunities for training and professional development has the project provided?
Nothing to report.

3d. What were the results disseminated to communities of interest?
Nothing to report.

3d. What do you plan to do during the next reporting period to accomplish the goals?
Frist, we will continue to develop cell lines expressing high or low levels of APOBEC proteins. Cell line pairs that are colored with GFP and YFP will be generated. This would conclude the phase 1 of this project.

Second, we will use the cell lines to study how ATR and DNA repair pathways respond to APOBEC induced DNA damage. We expect to identify DNA repair proteins important for the survival of cancer cells in the presence of APOBEC proteins. These studies (phase 2) will advance significantly in year 2 and be completed in year 3.

Finally, we will start the chemical screen in year 2. The initial screen will be carried in year 2, and it will be continued into year 3. We expect to obtain preliminary results from the screen in year 2.

4. Impact

4a. What was the impact on the development of the principal discipline(s) of the project?
We preliminary studies have identified a new way to express APOBEC proteins in E. coli, overcoming the toxicity problem for the research of APOBEC family members. Furthermore, our preliminary results have revealed for the first time that the ATR kinase is critical for the survival of cancer cells in the presence of APOBEC proteins. These findings may have important implications for the treatment of APOBEC overexpressing breast cancers.

4b. What was the impact on other disciplines?
Nothing to report.

4c. What was the impact on technology transfer?
Nothing to report.

4d. What was the impact on society beyond science and technology?
Nothing to report.

5. Changes/Problems

5a. Changes in approach and reasons for change
We proposed to focus on APOBEC3B in our proposal because APOBEC3B was found to be overexpressed in a large fraction of breast cancers. Recent studies have found that APOBEC3A was also overexpressed in several types of cancers, including ovarian cancer. We have included both APOBEC3A and APOBEC3B in our studies to extend the impact of our project.

In Task 2, we have used an alternative strategy to test if APOBEC overexpressing cells are defective for HR. As described above, RAD51 foci and the DR-GFP assay have caveats. In contrast, PARP inhibitor sensitivity has recently become a commonly used and reliable assay for HR defects. Using this new approach, we found that APOBEC overexpressing cells are not defective for HR.

5b. Actual or anticipated problems or delays and actions or plans to resolve them
There is not substantial problems or delays in our ongoing studies. We planned to initiate the chemical screen in year 1-2. The initial screen will now be carried out in year 2.

5c. Changes that had a significant impact on expenditures
None.

5d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
None.

6. Products

6a. Journal publications
None.

6b. Books or other non-periodical, one-time publications
None.

6c. Other publications, conference papers, and presentations
None.

6d. Websites or other internet sites
None.

6e. Technologies or techniques
None.

6f. Inventions, patent applications, and/or licenses
None.

6g. Other products
None.

7. Participants & Other Collaborating Organizations

7a. What individuals have worked on the project?

Name	Lee Zou
Project role	PI
Researcher identifier (ORCID ID)	0000-0003-3094-1058
Nearest person month	1
Contribution to project	Dr. Zou oversees the entire project
Funding support	

Name	Remi Buisson
Project role	Postdoctoral fellow
Researcher identifier (ORCID ID)	
Nearest person month	3
Contribution to project	Dr. Buisson contributed to the generation and testing o the APOBEC3A/3B expressing cell lines.
Funding support	The Tosteson Postdoctoral Fellowship

Name	David Moquin
Project role	Postdoctoral fellow
Researcher identifier (ORCID ID)	
Nearest person month	9
Contribution to project	Dr. Moquin contributed to the generation and testing of th APOBEC3A/3B expressing cell lines
Funding support	

Name	Alexander Hallet
Project role	Technician
Researcher identifier (ORCID ID)	
Nearest person month	2
Contribution to project	Mr. Hallet assisted Drs. Buisson and Moquin in thei experiments.
Funding support	

Name	Dominick Matos
Project role	Graduate Student
Researcher identifier (ORCID ID)	
Nearest person month	6
Contribution to project	Mr. Matos contributed to the generation and testing of th APOBEC3A/3B expressing cell lines.
Funding support	

7b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Changes of active other support of PI:

Exceptional Project Grant, Zou (PI)
Breast Cancer Alliance
Feb. 1, 2015 - Jan. 31, 2016
Total direct costs: $92,593
Title: Targeting the DNA repair addiction of APOBEC3B-overexpressing breast cancer
This project uses a new approach (ubiquitin-regulated APOBEC3B) to follow the recovery of APOBEC3B-expressing cells. There is no experimental overlap with the current project.

7c. What other organizations were involved as partners?
Nothing to report.

8. Special Reporting Requirements
Non-applicable

9. Appendices
None.